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Full Length Research Paper

Probiotic Strains Used in Aquaculture

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ABSTRACT

Aquaculture is the fastest growing animal food producing agricultural sector in the world which has been constrained by several factors. Infectious disease is a key limitations to the sustainable development of aquaculture sector. This paper reviewed the application and efficiency of probiotic microorganisms in aquaculture to prevent infectious diseases and their effects on fish immunology. Information was collected from different secondary sources like journals, reports, articles and electronic media and arranged chronologically. The study reveals that, currently, many probiotic products extracted from different species of bacteria including *Bacillus sp., Lactobacillus sp., Carnobacterium sp., Enterococcus sp.* and a yeast *Saccharomyces cerevisiae* are commercially available for use in aquaculture. Probiotics have gained popularity in aquaculture as an alternative to the antibiotic in the prevention of infectious diseases of fish because probiotics are eco-friendly. This review also shows that, probiotics have significant effect on growth rate, feed utilization capacity and the immune performance of finfish, shrimp, prawns and crabs in aquaculture. Probiotics are able to interfere with colonization of harmful pathogens and to improve the water quality through decomposition of uneaten food materials, nitrate and other organic materials.

Keywords: Aquaculture, food security, infectious disease, immunity, probiotics, microencapsulation.

Abbreviations: SGR: Specific Growth Rate; FCR: Food Conversion Ratio; PER: Protein Efficiency Ratio; WSSV: White Spot Syndrome Virus; IgM: Immunoglobulin M; APS: Astragalus Polysaccharide; NASS: National Agricultural Statistics Service; USDA: United States Department of Agriculture; FISH: Fluorescent in Situ Hybridization.

INTRODUCTION

Aquaculture is the fastest growing and most promising food producing agricultural sector which contributes almost half to the global fish production. It also provides employment opportunities and great contribution to the human food security and socio-economic development in many countries (FAO, 2008). Sustainable development of aquaculture have to face many challenges like disease prevention, improving resistance to pathogens, increasing growth performance and immunomodulation. Infectious diseases are the key threats to aquaculture which can result in economic loos causing high mortality in farmed fishes (Assefa and Abunna, 2018). Almost 90% production loss was caused by disease in rainbow trout (*Oncorhynchus mykiss*) culture in 2009 (NASS, 2010). A major tool in prevention of diseases in fish, shrimp and crab aquaculture was antibiotics until now; but treating infectious diseases with antibiotics have been proven unsustainable and ineffective as pathogens can develop resistance against antibiotics (Ayisi et al., 2017). This problem demands for alternatives of antibiotic for disease control in aquaculture. Application of probiotics in combination with prebiotics or alone have been reported to be a suitable alternative of antibiotics, because biological control is the best approaches against infectious disease (Maqsood et al., 2011).

Host species cannot utilize many important nutrients, but the gut microbiota can metabolize and convert them to end products like short-chain fatty acids. So, modulation of the intestinal microbiota of aquatic organisms in a positive way is very important which can be done administering probiotics (Wang et al., 2008; Merrifield et al., 2010; Dimitroglou et al., 2011; Gioacchini et al., 2014; Ringo et al., 2014). Probiotics are the beneficial microorganisms including bacteria and yeast which confers a health benefit to the host when administered in adequate amount (WHO, 2002). Probiotics are bioactive, non-digestible and fermentable food materials which provide benefits to the host animal through stimulation of its growth and activity of internal microorganisms (Ayisi, 2017). Probiotics are beneficial for the hosts, they are able to persist in the digestive tract because of their tolerance to acid and bile salts (Cruz et al., 2012). The use of probiotics has gained increasing scientific and commercial interest and are now quite commonplace in health - promoting foods to therapeutic, prophylactic and growth supplements (Nayak, 2010; Kiron, 2012; Ringo et al., 2014). It has been reported that, Probiotics have important beneficial effects on aquatic animals like increasing disease resistance and nutrient availability (Merrifield et al., 2010; Carnevali et al., 2014; Ringo et al., 2014). This review was conducted to investigate the application and efficiency of probiotic microorganisms and their products in aquaculture to control infectious diseases of fish and increasing immune response to the harmful pathogens. This paper also reviewed the probiotic products currently available in the market for use in aquaculture for the prevention and control of infectious fish diseases.

REVIEW OF FINDINGS

Probiotics are alive or dead whole microorganism or part of a microorganism or extract of microorganism which confers health benefits to the host when administered at appropriate dose and improve disease resistance, growth performance, feed conversion ratio (FCR) and stress resistance (Hoseinifar et al., 2014). The origin of using of probiotics in aquaculture is not clear from the historical perspective and available literatures but, evidence exists on usage of probiotics in extensive finfish and invertebrate culture in China and India. It has been reported that, the word probiotics was first used by Lilly and Stillwell in 1965 to denote health promoting bacteria which was then defined by Fuller as a live microbial feed supplement having ability to improve the microbial balance of host animal in 1989 (Balcazar et al., 2006; Austin et al., 2012).

Sources of probiotics

Westerdahl et al. (1991) preferred the host-derived micro-organisms as probiotics to be used against pathogen. Probiotic microorganisms may be derived from intestine or guts of healthy fish, water of rearing environment, sediments of culture tank, other animals, and different fermented food products. The efficacy of commercial probiotic is not clear from the literature stock but host-derived probiotics were found beneficial to host microbiota isolated from the gastrointestinal tract of aquatic (Hai, 2015). Those microorganisms are beneficial which lives inside a healthy hosts and they are argued to be the essential part of natural defense system (Gomez et al., 2013). Terrestrial microorganisms were also proven ineffective as probiotic for marine organisms (Vazquez et al., 2003). *Lactococcus lactis, Lactobacillus brevis, Lactobacillus collinoides, Lactobacillus coryniformis, Enterococcus faecalis, Citrobacter freundi, Lactobacillus farciminis, Lysinibacillus fusiformis, Pseudomonas fluorescens, Bacillus circulans, Enterococcus durans, Streptococcus sp. I, Streptococcus sp. II, Leuconostoc sp., Enterococcus faecium* and many other potential probiotics have been isolated from *Oreochromis niloticus* (Reda et al., 2017). *Bacillus licheniformis, Enterococcus faecalis, Bacillus pumilus, Enterococcus faecium, Lactobacillus lactis, Bacillus subtilis* were isolated from Rainbow trout and *Mugil cephalus* having high potential to be used as aquaculture probiotics (Hai, 2015).

Administration strategies

Immunomodulatory activity of probiotics depends on various factors like source of probiotic, dose of probiotic, method of administration and the duration of supplementation (Hai, 2015). Proper administration method is a key factor to use the probiotics in aquaculture.

- Dietary Administration: Direct incorporation of probiotic in pelleted feed is one of the most important and widely applicable administration of probiotics. Probiotics are directly applied in the form of spore with feed pellets (Assefa and Abunna, 2018). During the addition of probiotics, the viability should be checked continuously to confirm protective enhanced immunity in the fish. They can be added as freeze-dried cultures that can be mixed with lipids as top dressings in the feed (De et al., 2014).
- Microencapsulation: The widely used administration strategy is encapsulation. Encapsulation helps by improving nutritional values and proper delivery of the microbe to the host (Assefa and Abunna, 2018). In this process, probiotic strains at high density are encapsulated in a colloidal matrix using alginate, chitosan, carboxymethylcellulose, or pectin to physically and chemically protect the microorganisms (Hermosillo et al., 2012). In aquaculture, application of Shewanella putrefaciens was done by encapsulating in calcium alginate which demonstrated the survival of encapsulated probiotic cells through the gastrointestinal tract of sole, Solea senegalensis. Encapsulation in alginate

matrices protects bacteria from low pH and digestive enzymes (Kumar et al., 2016).

- Immobilized Probiotics: Immobilization of Probiotic is a new technique, extensively used in dairy and pharmaceutical industries which has been reported to be advantageous (Assefa and Abunna, 2018). It is a new technology, extensively used in the dairy and pharmaceutical industries, applied to Lactobacillus species. In addition, cell immobilization offers many advantages for biomass and metabolite production compared with free cell systems (De et al., 2014).

Application of probiotics

Probiotics can be applied in different form such as using only Probiotic strains, Probiotics with plant products and Probiotics with yeast extracts. Using probiotics with plant product is a promising disease control approach which can improve growth performance, haematological parameters, immune response and disease resistance of fish (Ringo and Song, 2015). But, little information is available on the effect of plant-probiotic mixture in aquaculture (Ringo and Song, 2015).

Using Only Probiotics: *Carnobacterium sp.* successfully reduced the diseases of salmonids caused by *Vibrio ordalii, Aeromonas salmonicida* and *Yersinia ruckeri* (Robertson et al., 2000); *Vibrio anguillarum* improved disease resistance of cod fry; multi-strain probiotics provide synergistic beneficial effects on the host health. A mixture of *Bacillus subtilis* and *Lactobacillus acidophilus* provided higher protection against harmful pathogens in tilapia such as *Aeromonas hydrophila, Pseudomonas fluorescens* and others (Sharifuzzaman and Austin, 2017).

Probiotics with Plant Extracts: Supplementation of *Lactobacillus sakei* and *Scutellaria baicalensis* plant extract (Harikrishnan et al., 2011), *Lactobacillus plantarum* with polysaccharide and *Lactobacillus plantarum* with *Helianthus tuberosus agar* (Van Doan et al., 2014; Ringo and Song, 2015), *Bacillus sp.* and a mixture herb extracts (Yu et al., 2009), mixture of *Pediococcus parvulus, Candida parapsilosis* (yeast) and *Echinacea purpurea* and *Uncaria tomentosa* (Peraza-Gomez et al., 2014) and many others have been reported to increase growth rate, FCR, PER, SGR and disease resistance in fish and shellfish.

Probiotics with Yeast: Probiotic bacteria *Bacillus licheniformis* with yeast extract significantly (Hassaan et al., 2014), *Shewanella putrefaciens* with β-glucans (Guzman-Villanueva et al., 2014); Vibrio alginolyticus with β-glucans (Fan et al., 2010; Ringo and Song, 2015) and their combinations increased immune response, SGR, FCR, PER, survival rate, serum IgM level, anti-protease activity and phagocytic activity in Nile tilapia, shrimp and gilthead seabream. Injecting *Bacillus subtilis* with APS and Tuckahoe improved SGR, immune response and disease resistance against *Vibrio splendidus* (Sharifuzzaman and Austin, 2017).

Mechanism of actions

The mechanisms of action of probiotics were not completely understood. Probiotics can expel out harmful pathogens after entering the digestive tract of host, the beneficial micro - organism produce inhibitory molecules, compete with pathogens for binding site, nutrient, or energy and interfere with pathogen activities (Balcazar et al., 2006; Irianto and Austin, 2002). Some are capable to prevent pathogen growth on the gut surface. Addition of Lactobacillus lactis, L. plantarum and L. fermentum reduced Aeromonas salmonicida, A. hydrophila, Vibrio anguillarum and Yersinia ruckeri in the intestinal mucus of rainbow trout (Balcazar et al., 2006). Mixture of Bacillus subtilis and Bacillus licheniformis significantly improved of FCR, SGR and PER in rainbow trout (Merrifield et al., 2010). The mechanisms of action of bacteria used as probiotics, although not yet fully elucidated, are described as following (Balcбzar et al., 2006; Ng et al., 2008; Walker 2008; Sherman et al., 2009):

- Competition for binding sites: It is also known as "competitive exclusion", where probiotic bacteria bind with the binding sites in the intestinal mucosa, forming a physical barrier, preventing the connection by pathogenic bacteria.

- Production of antibacterial substances: Probiotic bacteria synthesize compounds like hydrogen peroxide and bacteriocins, which have antibacterial action on the pathogenic bacteria. They also produce organic acids that lower the environment's pH of the gastrointestinal tract, preventing the growth of various pathogens and development of certain species of Lactobacillus.

- Competition for nutrients: Probiotics completes with the harmful pathogens for nutrition absorption which reduces the amount for nutrients. These lack of nutrients available that may be used by pathogenic bacteria is a limiting factor for their maintenance.

- Stimulation of immune system: Some probiotics bacteria are directly linked to the stimulation of the immune response, by increasing the production of antibodies, activation of macrophages, T-cell proliferation and production of interferon.

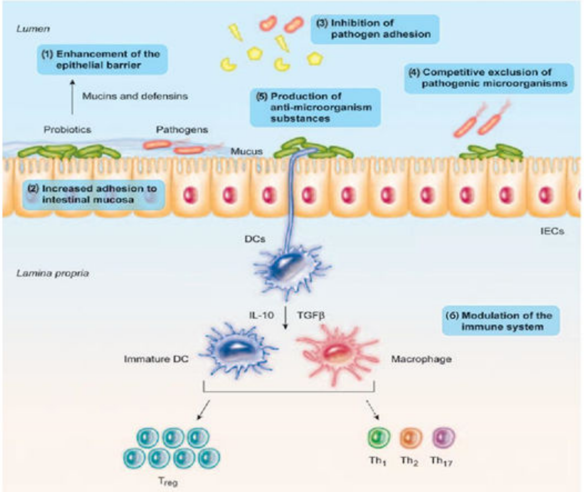

Figure 1. Mechanisms of action of Probiotics in Fish and other organisms (Bermudez-Brito et al., 2012)

Effects of probiotics

Probiotics are used in aquaculture to increase the growth performance of cultured species, to increase the appetite and digestibility of fish. Probiotics have the capacity to control diseases, promote growth rate, acts as source of nutrients, contribute in feed digestion, and improve the immune response. Probiotic are promising approach to inhibit the virulence of pathogens and to control the diseases in aquaculture species. Probiotics are capable to release chemical substances having bactericidal or bacteriostatic effect (De et al., 2014). Probiotics have important beneficial effects on the digestive system of fish. Probiotic can synthesize enzymes like amylase, proteases and lipases. Nutrients absorption capacity increases when probiotics are added to the feed in appropriate dose (Merrifield et al., 2010). High level of probiotics in fish ponds can minimize the accumulation of dissolved and particulate matters in the growing season and balance the production of phytoplankton (Ibrahem, 2015). It has been reported that, Probiotics increased stress tolerance in intensive aquaculture zebra fish (Danio rerio) (Cruz et al., 2012). Many of the aquatic microbes have been proven to enhance disease resistance in fish and shellfish species against multiple pathogens (Newaj-Fyzul et al., 2015). Prolonged administrations of probiotics induced immune responses of hosts, improved health status, improved disease resistance and increased growth rate. Swain et al. (2009) fed the *Penaeus monodon* with four probiotics and concluded that the probiotic strains effectively inhibited the pathogens, increased survival rate to *Vibrio harveyi* and disease resistance against *Vibrio parahaemolyticus*. He proved that the *Streptococcus phocae* and *Enterococcus faecium* isolated from brackish water shrimp is highly potential to control pathogenic vibriosis in shrimp culture (Swain et al., 2009). A LAB strain *Lactobacillus plantarum* MRO3.12 showed the highest efficiency in reducing *Vibrio harveyi* pathogen. Supplemented diet containing *Lactobacillus plantarum* showed (L.) significantly increased relative growth rate (RGR), feed conversion ratio (FCR) and survival rate of *Litopenaeus vannamei* (Kongnum and Hongpattarakere, 2012). Nimrat et al., (2012) examined the effectiveness of mixed Bacillus probiotics and mode of action on growth, bacterial numbers and water quality in *Litopenaeus vannamei* and found that the Post larvae treated with probiotic exhibited higher growth, high survival of shrimp, increased beneficial bacteria and enhanced water quality (Nimrat et al., 2012).

Available commercial probiotics

A lot of probiotic microorganisms have been isolated and evaluated for use in aquaculture in prevention and control of infectious diseases of aquaculture species. Some of these have been isolated from aquatic species including finfish and shrimps, their intestines and guts, culture environment, terrestrial animals and other sources.

Table 1. Commercially available Probiotic products for use in aquaculture (Balcazar et al., 2006; Merrifield et al., 2010; Nayak, 2010; Cruz et al., 2012; Ringш et al., 2014; Hai, 2015; Ibrahem, 2015).

SL. No.	Microorganisms	Target Species
1	*Bacillus sp.*	Catfish, Penaeids
2	*Carnobacterium divergens*	*Gadus morhua*
3	*Alteromonas sp.*	*Crassostrea gigas*
4	*Lactobacillus helveticus*	*Scophthalmus maximus*
5	*Lactobacillus lactis*	*Brachionus plicatilis*
6	*Streptococcus thermophilus*	*Scophthalmus maximus*
7	*Streptomyces*	*Xiphophorus helleri*
8	*Lactobacillus casei*	*Poeciliopsis gracilis*
9	*Bacillus sp. Vibrio sp.*	*Macrobrachium rosenbergii*
10	*Bacillus coagulans*	*Cyprinus carpio*, koi
11	*Enterococcus faecium*	*Anguilla anguilla*
12	*Lactobacillus rhamnosus*	*Oncorhynchus mykiss*
13	*Pseudomonas fluorescens*	*Oncorhynchus mykiss*
14	*Pseudomonas sp.*	*Oncorhynchus mykiss*
15	*Roseobacter sp.*	Scallop larvae
16	*Saccharomyces cerevisiae*	*Litopenaeus vannamei*
17	*Phaffia rhodozyma*	*Litopenaeus vannamei*
18	*Vibrio alginolyticus*	Salmonids

19	*Vibrio fluvialis*	*Oncorhynchus mykiss*
20	*Tetraselmis suecica*	Salmo salar
21	*Carnobacterium sp.*	*Hepialus gonggaensis*
22	*Lactobacillus acidophilus*	*Clarias gariepinus*
23	*Bacillus spp.*	*Farfantepenaeus brasiliensis*
24	*Enterococcus sp.*	*Farfantepenaeus brasiliensis*
25	*Lactococcus lactis*	*Epinephelus coioides*
26	*Lactococcus helveticus*	*Scophthalmus maximus*
27	*Bacillus sp. and Vibrio sp.*	*Macrobrachium rosenbergii*
28	*Carnobacterium sp.*	*Hepialus gonggaensis*
29	*Lactobacillus acidophilus*	*Clarias gariepinus*
30	*Shewanella putrefaciens*	*Solea senegalensis*
31	*Bacillus sp.*	*Penaeus monodon*
32	*Lactobacillus acidophilus*	*Clarias gariepinus*
33	*Bacillus coagulans*	*Pennaeus vannamei*
34	*Lactobacillus delbrueckii*	*Dicentrarchus labrax*
35	*Saccharomyces sp.*	*Penaeus monodon*
36	*Bacillus subtilis*	*Paralichthys olivaceus*
37	*Pediococcus acidilactici*	*Litopenaeus stylirostris*
38	*Bacillus subtilis*	*Poecilia reticulata*
39	*Lactobacillus rhamnosus*	*Danio rerio*
40	*Lactobacillus acidophilus*	*Xiphophorus helleri*
41	*Lactobacillus casei*	*Xiphophorus helleri*

Limitations and prospects

Probiotic products activate the defense mechanism and innate immunity of host, influence the virulence of disease causing pathogen and stimulate the response to stressors, but these aspects have been investigated very less for sturgeons (Askarian et al., 2011; Ringo and Song, 2015). The possible mechanism of action of probiotics largely depend on probiotic–host interactions (Sharifuzzaman and Austin, 2017). But, there is uncertainties about the longevity of protection conferred by probiotics (Newaj-Fyzul and Austin, 2015). To date, aquaculture studies on probiotics in combination with plant products and b - glucan, have not investigated properly, the effect on epithelial barrier function, gut integrity and architecture, disease resistance against pathogenic bacteria (Sissener et al., 2009). Successful formulation of probiotics in combination with plant products and b-glucan is a complex issue and a little information is available from aquaculture studies (Gatlin et al., 2007). The impacts of probiotic strain or probiotic products on tilapia (*Oreochromis niloticus*) aquaculture has not been fully understood. Besides, commercially-available probiotic microorganisms do not provide beneficial effects on Nile tilapia (Hai, 2015). The combined use of probiotics and plant products is in its infancy, so the topic deserves further attention. An aspect that deserves attention is the dietary supplement, combination of probiotic bacteria and plant-based replacers in aqua feeds, as the continued growth and intensification of aquaculture has increased inclusion of plant based replacers in diets (Hansen et al., 2015). Further investigations on these issues alongside an environmental impact assessment of probiotics are key requirements to realize desirable outcomes in aquaculture. Clearly, there is a role for probiotics in fish and shellfish disease control strategies, and their use can not only help to replace some of the inhibitory chemicals currently used in aquaculture but also promote food and health safety (Sharifuzzaman and Austin, 2017).

CONCLUSION

The probiotics in aquatic environment is still a controversial concept due to lack of authentic evidence or real environment demonstrations on the successful use of probiotics and their mechanisms of action. Probiotic is an alternative to antibiotics and chemicals in aquaculture which provide better health benefits, higher growth rate, increased survival rates and produce safe organic fish products to meet the protein requirements of future generations. Further research on probiotics should focus on molecular biotechnology tools to gain a greater understanding of the modes of action because the exact mode of action of probiotics is not totally known in fish. Using immunohistochemistry, gene expression and proteomics can be used to explore the mechanisms of actions of probiotics. Researches on the interaction between probiotics and carbohydrate-to-lipid ratio can reduce pressures on feed nutrition in the intensive aquaculture industry. The FISH technique is a potential tool to characterize the dynamics of potential probiotic bacteria and their efficiency in the control of pathogenic bacteria in pathogen detection.

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Conflicts of Interest

The author of this article declares that there is conflicts of interest or financial interests regarding the publication of this article.

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